General discussion

Mexican sunflower is an exotic plant which has been introduced to grow in several tropical countries including Thailand. This plant is able to adapt and succeed to establish, and become a dominant plant in some mountainous areas in the northern part of Thailand. The succession of Mexican sunflower was hypothesized to be related to its allelopathic interaction to other plants. The primary goal of this research was to find the evidences of allelopathic activity of Mexican sunflower and to determine the factors affecting production, release and allelopathic activity of this plant in soil. This research was done in order to obtain the basic information on the allelopathic potential of Mexican sunflower under natural conditions that may be valuable for developing better weed management in sustainable agriculture.

In this study, several experiments were conducted using bioassays as the primary tool to test and compare the phytotoxic activity of Mexican sunflower. Water was selected to be used as the solvent for extracting the phytotoxic compound(s) because the phytotoxic substance(s) in the plant would be washed out by rain from the upper parts and extracted with water from the underground parts in soil under natural conditions. The study in chapter 1 demonstrated that water extract from Mexican sunflower leaf powder at the concentration of 20 mg DME/ml

significantly inhibited seed germination and seedling growth of most test plant species in the petri dish bioassay. The degree of inhibition varied depending on the concentration of the extract and plant tested. Water extract from various parts of this plant including mature leaves, senescent leaves, stems and roots could inhibit seedling growth of test plant. These results suggested that Mexican sunflower contained water soluble plant growth inhibitor(s) which have a capacity to reduce seed germination and seedling growth of other plants. This may not exclude the presence of water-insoluble or organic solvent extractable phytotoxic compounds. Water-insoluble compounds may be decomposed or changed to water soluble phytotoxic ones in soil or plant bodies when time elapsed. Baruah *et al.* (1994) reported that tagitinin A, tagitinin C and hispidulin, which were isolated from Mexican sunflower leaves by organic solvent extraction, reduced seed germination and seedling growth of radish, cucumber and onion in petri dish bioassay but their phytotoxic activity on the growth of plant in soil have not been examined yet. In the present study, the contents of Mexican sunflower leaves were extracted with water and their phytotoxic activity was investigated on the germination and growth of several plant species in both petri dish bioassay and soil bioassay. The study using water extract and bioassay in the soil may obtain results more closely related to natural field conditions.

The difference in phytotoxicity of the extracts from various

plant tissues have been reported previously (Mersie and Singh, 1987; Putnam and Tang, 1986). Such difference might be related to phytotoxic compounds being produced in large quantities and/or high activities in certain tissue which provides a higher level of phytotoxic activity (May and Ash, 1990). The water extracts from the leaves and stems of Mexican sunflower showed a greater inhibitory effect on plant seedling growth compared to the water extract from the roots. This suggested that the phytotoxic compound(s) may be produced in larger quantities in the leaves and stems than in the roots. Moreover, both the leaves and stems may be the major sources of the phytotoxic substance(s) of this plant in natural fields.

Heisey (1990) emphasized that, although the phytotoxic substances are present in various plant parts, their presence may not establish allelopathy. In order to prove their involvement in allelopathy, it is important to find their direct release into the environment and the existence of chemicals in sufficient quantities and remaining for a sufficient time in the soil to affect other organisms (Putnam and Tang, 1986). The study in chapter 2 demonstrated that soil previously planted with Mexican sunflower for six months contained phytotoxic compound(s) in a sufficient amount to inhibit seedling growth of other plant species. Mexican sunflower leaf powder incorporated into soil and its water extract at the concentrations of 10 to 20 mg DME/g dry soil applied to soil could inhibit shoot and root growth of the tested

plant. The residual phytotoxic effect of water extract and leaf powder residue were present in soil for 4 weeks or longer. These facts suggested that the growth inhibitory substance(s) released from Mexican sunflower leaves have phytotoxic activity in soil and the activity could be present in the soil for a period sufficient enough to affect the growth of other plant seedlings. This finding suggests the allelopathic potential of Mexican sunflower under natural conditions. In this experiment, no attempt was made to isolate and use the pure active compounds of tagitinin A, tagitinin C and hispidulin for bioassay because they are very difficult to obtain in sufficient amounts. However, by using the water extract from Mexican sunflower parts instead of the purified compounds, it is possible to suggest that Mexican sunflower plant contains phytotoxic substance(s) which should be extracted under natural fields conditions. Further studies are needed to clarify that the phytotoxic activity of Mexican sunflower in soil is actually induced by which individual or combined action of these three phytotoxic compounds and/or the other water extractable substances.

The phytotoxic effect of allelopathic plant in soil have been demonstrated to be related to the concentration of allelochemical in soil-water (Ito *et al.*, 1998). The study in chapter 2 demonstrated that soil-water separated from the soil previously planted with Mexican sunflower, from the soil incorporated with Mexican sunflower leaf powder and from the soil applied with its

water extract could inhibit seedling growth of the tested plant in a similar extent to those in the planted soils or in the applied soils. This suggests that the phytotoxic effect of Mexican sunflower in soil depends on the concentration of phytotoxic compound(s) in soil-water which was released from the living plant and its leaf residue in soil and easily absorbed through plant roots, although the active compound(s) as described above was not identified and determined in the present study.

There are many factors affecting the phytotoxic activity of allelochemicals in soil. Several literatures reported that soil factors such as soil moisture, texture, organic matter, clay contents and microorganisms influence quantitative and qualitative availability of plant allelochemicals in soil (Blum, 1995; Cheng, 1995; Dalton *et al.*, 1983; Inderjit, 1996b; Inderjit and Dakshini, 1994a; Teasdale, 1993; Yun and Kil, 1992; Zhang, 1993). The study in chapter 3 demonstrated that the phytotoxic activity of water extract from Mexican sunflower leaf powder on the growth of rice seedlings used as bioassay plant varied among the three test soils which possess different physical and chemical properties. The phytotoxic activity of the extract seemed to be greater in Ryugasaki soil, which contained lower organic matter and clay content, than the activity in Kannondai soil and Yawara soil, which have more organic matter and clay. This suggested that the phytotoxic activity of Mexican sunflower in soil differed depending on the soil properties. The inhibitory effect of water

extract from Mexican sunflower leaf powder decreased after application into soil. This suggested that soil could adsorb the phytotoxic substance(s) from the extract, resulting in a reduction of the inhibitory activity. The possibility that phytotoxic activity of allelochemicals could be reduced in soil through soil adsorption has been widely considered in several documents (Inderjit and Dakshini, 1995b; Lovett, 1982; Putnam and Tang, 1986; Rice, 1987) but the evidences supporting this assumption have not been clearly reported yet as pointed out by Ito *et al.* (1998). In the present study, the phytotoxic activity of water extract from Mexican sunflower leaves on the growth of rice seedling was compared in the soil and sea sand cultures, and the inhibitory activity of the leaf extract was also compared with that of the soil-water immediately separated from the soil applied with the extract. The results clearly showed that the phytotoxic activity of water extract from Mexican sunflower leaves was reduced after application into the soil.

Soil microbes have been reported to be an important factor influencing the allelopathic effect of allelochemicals in soil (Chase *et al.*, 1991). The study in chapter 2 showed that the phytotoxic activity of Mexican sunflower leaf residue and water extract of leaf powder applied into soil was reduced with time after application. The investigation in chapter 3 found that the phytotoxic activity of water extract from Mexican sunflower leaf powder applied into non-autoclaved soil was less than that

applied into autoclaved soil. These suggested that soil microorganisms degraded phytotoxic substance(s) in soil, causing a reduction in inhibitory activity. There is the possibility that some kinds or amounts of soil organic matters could be changed into non-organic substances after autoclaving at high temperature and high pressure. This alteration may directly or indirectly affect the phytotoxic activity of chemicals contained in water extract from Mexican sunflower leaves in the autoclaved soil, but it is believed that the reduction in phytotoxic activity of the extract in non-autoclaved soil compared to that in the autoclaved soil was mainly related to the degradation of the phytotoxic compounds by soil microorganisms rather than the difference in the amount and kind of organic and non-organic substances between the autoclaved and non-autoclaved soils.

Most allelochemicals are released into soil through water leaching directly from living plant by rain and by dissolution of the plant residue in soil (Lovett and Hoult, 1995; Nelson, 1996). The study in chapter 4 demonstrated that water leachate from Mexican sunflower leaves obtained by water spray have a phytotoxic effect on the growth of test plant in soil. This suggested that phytotoxic substance(s) in Mexican sunflower leaves could be eluted by rain and has a potential to inhibit the growth of other plant under natural conditions.

Water movement also affects the distribution of plant allelochemicals in soil (Inderjit and Dakshini, 1996). The study in

chapter 5 demonstrated that the phytotoxic substances in soil treated with water extract from Mexican sunflower leaves could move down with water to the lower layer of the soil column, and the phytotoxic activity of each soil layer of the soil column applied with water extract varied. Phytotoxic activity also differed between the wet and dry conditions in the soil column before water extract was applied. This suggested that the mobility of the phytotoxic substances in soil are influenced by water movement and soil moisture condition, although the movement mechanism should be investigated.

It has been reported that environmental stress affects the production and the phytotoxic activity of plant allelochemicals (Chaves *et al.*, 1997; Inderjit, 1996b; Penuelas and Llusia, 1997). The most common environmental stress on plants is probably water stress (Tang *et al.*, 1995). The study in chapter 6 suggested that water stress not only directly affects the growth of Mexican sunflower but also enhances the production of phytotoxic compound(s) in the leaves, stem and roots of this plant. According to these findings, the proposed processes which the phytotoxic compound(s) are released from Mexican sunflower plant and its residues into soil and the factors involving its phytotoxic activity in soil could be summarized as shown in Fig. 7.1. Since there are several rainfall events during the rainy season, the compound(s) contained in the whole plant of Mexican sunflower and its residues could be eluted from living leaves, stems, dead plants

and these residues into soil by repeated rain washing. In addition, the phytotoxic compound(s) are also continuously released into soil through the exudation from roots and the decay of root tissues in soil. Once the phytotoxic compounds of Mexican sunflower are released into soil, many physical, chemical and biological processes affect the activity of these compounds in soil. The phytotoxic compounds could be adsorbed onto soil particles and degraded either by soil microorganisms and/or other chemical reactions. Some amounts of them might be moved with water into the deeper soil layer and might be washed out from the top soil by water leaching or through surface run off. The phytotoxic effect of Mexican sunflower in soil seems to relate to the amount or the concentration of the phytotoxic compound(s) in the soil-water. Afterwards the seeds or seedling of plants absorbed the substance(s) in the soil-water, resulting in the phytotoxic effects on seed germination and/or seedling growth of the susceptible plants. Although this schematic is a simplified view and not a full spectrum cover of all factors affecting the phytotoxic activity of this plant, this illustration may be valuable for determining the allelopathic potential of Mexican sunflower under natural field conditions.

Zungsontiporn and Harada (1995) reported that Mexican sunflower is a herbaceous perennial plant which can grow very fast in the mountainous rainfed areas of some provinces in the northern part of Thailand. In Mexican sunflower infested areas,

it was found that during 5 months of rainy season, this plant produces green and senescent leaves about 80-300 and 25-150 g per m^2, respectively, depending on age, plant density and soil fertility. The living and senescent leaves are the major parts of the plant and most of the fallen leaves and dead plant remain on the soil surface. In that area, the rainy season starts from June until the end of October. The average amount of rainfall in Mexican sunflower growing areas is 950 mm with 32 rainy days in that particular season. This amount of rainfall is estimated to be sufficient to leached the phytotoxic compound(s) from the plant and its residues into soil in a concentration sufficient to inhibit germination of other plant seeds, which are mostly located in the top 5 cm of the soil layer. Certainly, some of them could germinate but the seedlings may absorb the phytotoxic substance(s) and their growth is thus inhibited. Although the seedlings are not killed by the phytotoxic compound(s), the suppression of growth at the seedling stage due to competition with Mexican sunflower plants should be taken into consideration as another important way of defeating the neighboring plants. Under natural field conditions, when Mexican sunflower has been grown in the first year, it is assumed that the plant would also be in competition with other plant species and the phytotoxic activity on the growth of neighboring plants could not be observed. However, according to the fast growing characteristic and the perennial life cycle of Mexican

sunflower, it has the ability to compete and continually grow in the second year. At this period, Mexican sunflower may produce and release phytotoxic compounds into the soil in amounts sufficient to reduce germination and suppress seedling growth of other plants. In the following years, plant density and canopy of Mexican sunflower increases rapidly, so that the high populations of Mexican sunflower not only enhance the allelopathic activity but also increase their competitive ability against other plants. These two combined effects may be related to the establishment of Mexican sunflower community under natural field conditions.

There are several documents showing that some allelopathic plants have autotoxicity (Chou, 1995a; Chung and Miller, 1995a, 1995b; Nakamura and Nemoto, 1993; Waller *et al.*, 1995). Autotoxicity occurs when a plant releases phytotoxic substance(s) that inhibit germination and/or growth of the same plant species (Miller, 1996). In the present study, autotoxicity of Mexican sunflower has not been examined because the seeds have a very low germination rate that could not to be used in the study of autotoxicity. However, from observation in natural fields it was found that a lot of Mexican sunflower seedlings germinated from the seeds previously distributed under its canopy and these seeds were able to grow healthy in high density. Based on this observation, it is considerable that Mexican sunflower may have no autotoxicity.

During the dry season which has no rainfall, the soil

moisture at the top 10 cm of the soil profile in Mexican sunflower growing areas is about 20 % or lower on a dry soil weight basis. In such water stress condition, Mexican sunflower can survive but its growth is clearly reduced. However, the plant still produces phytotoxic compound(s) in all of its parts and releases the compound(s) into the soil through exudation from the roots. During the dry season, elution of phytotoxic substance(s) from the leaves and residues into soil is quite limited. However, if there is an occasional rainfall during this period, the phytotoxic compound(s) in the leaves and residues could easily be washed out and released into the soil by rain. Precipitation is the main factor involving the downward movement of allelochemicals (Weidenhamer, 1996). In the case of Mexican sunflower under natural field conditions, the phytotoxic substances could be eluted from the plant and its residues into soil by rain. However, during heavy rainfall events, the phytotoxic substance(s) in the top soil might be moved down to the lower soil layer by water movement. Thus, it is likely that the phytotoxic activity of the plant is a function of static availability and dynamic availability based on the total amount of compounds moving in and out of the system over a period of time. May and Ash (1990) reported that the concentration of allelochemicals in soil was evidently dependent not only on the amount of source material but also on water balance. It was assumed that low rainfall events, which are usually insufficient to cause runoff or deep drainage, will tend to

maximize allelochemical concentration in soil.

The environmental conditions, where the allelopathic plants are growing, directly modify growth and the rate of production of allelochemicals. In another perspective, the environmental conditions may affect the biological activity of allelochemicals (Einhellig, 1996). In the present research, the effects of soil factors and water movement on the phytotoxic activity of Mexican sunflower in soil and the effect of water stress on growth and phytotoxic activity of Mexican sunflower plant have been selected for study because these factors are important and commonly exist under natural field conditions. The areas where Mexican sunflower were growing in Thailand are mountainous with differences in slope, soil characteristics and existent plant species. It is assumed that the allelopathic activity of Mexican sunflower in these areas may be varied depending upon the environment in which it is grown. From the preliminary experiment in Thailand, it was found that the soil collected from Mexican sunflower infested areas showed inhibitory effects on seed germination and seedling growth of some crops and weeds such as rice (*Oryza sativa* L. cv. KDML 105, cv. Sew Mae Chun, cv. Nam Roo, crabgrass (*Digitaria ascendens* Henr.), spiny amaranth (*Amaranthus spinosus* L.) and painted spurge (*Euphobia geniculata* Ort.), but has no effect on the germination and seedling growth of maize (*Zey mays* L.), soybean (*Glysine max* Merrill.) and black bean (*Vigna sp.*) compared with those

grown in the same soil type collected from non-infested areas. Generally, soils in northern Thailand where Mexican sunflower have been grown are sandy loam or sandy clay loam with organic carbon lower than 1.0 % (Ogawa *et al.*, 1980). Table 7.1 shows the soil characteristics of Hang Chut soil which represents the physicochemical properties of soil in Mexican sunflower growing areas in Thailand. In the present study, the phytotoxic activity of water extract or leachate from Mexican sunflower leaves in Thai soils has not been investigated yet, however, based on the experiment using Japanese soils (Table 3.1) as materials for bioassay, it is considered that the phytotoxic activity of Mexican sunflower in Thai soils should occur more remarkably than the soils used in the present study because Thai soils have lower organic matter and clay contents compared to Japanese soils (Table 3.1 and Table 7.1). Also the degree of phytotoxic activity may vary depending on the soil properties and precipitation in those areas. Further studies on the effects of these environmental factors should be conducted in actual field conditions for several years to understand the realistic allelopathic activity of Mexican sunflower under natural conditions.

In this study, no attempts were made to determine the qualities and quantities of the phytotoxic compound(s) contained in water extract and soil-water, but it is believed that allelopathic activity of Mexican sunflower in soil is induced by the combined action of water soluble and/or non-water soluble phytotoxic

substance(s) such as tagitinin A, tagitinin C and hispidulin (Baruah *et al.*, 1994) in soil-water. Einhellig (1996) emphasized that it is an error to assume that there should be enough of a single compound present in a field situation to affect growth of a receiving plant. Inderjit (1996b) stressed that allelopathic interactions are mostly the result of the synergistic activity of allelochemicals rather than of a single compound. Under field conditions, additive or synergistic effects become a more influential event at low concentrations compared to the effect of individual compounds (Einhellig, 1996). Inderjit and Dakshini, (1996) reported that it is difficult to determine the exact biological concentrations of allelochemicals in soils under field conditions because the compounds have been determined through the input of leaf leachates, root exudates, decomposing and leaching from plant parts, adsorption by soil components and microbial activity, which are of uncertain distribution (Cheng, 1995; May and Ash, 1990). However, to understand the mechanism of action of allelochemicals in soil, the identification of compounds and the measurement of their concentrations in soil-water are most desirable.

Understanding on allelopathy of Mexican sunflower may be valuable in weed control management in the upland areas of northern Thailand. For this purpose, Mexican sunflower might be suitable in both non-cropping and cropping areas. In non-cropping areas, particularly in range land and deforested areas,

Mexican sunflower can be grown as the cover plant to prevent soil erosion and reduce weed infestation prior to the land being reforested. In non-cropping areas, Mexican sunflower might be used as a green manure plant. It can be mulched on the soil or incorporated into the soil for weed control and for improving soil fertility in some selected crops, such as maize, soybean, black bean or some vegetable crops that tolerate to the phytotoxic compound(s) contained in this plant. Proper management of Mexican sunflower residue in the cropping areas may help to improve upland crop production in the future.

In addition, according to the results in this research, they suggested that Mexican sunflower has a potential to cause a reduction of seedling growth of some crops. The planting of susceptible crops such as rice in the lower areas close to a Mexican sunflower community, should be avoided. The utilization of Mexican sunflower as a green manure or as a mulching plant to prevent soil erosion on the high hillside slopes should be carefully managed. More intensive investigations in Mexican sunflower growing areas are required before suggesting the appropriate management of this plant and the suitable cropping system in these areas.

Based on these results, it could be concluded that Mexican sunflower has allelopathic potential under natural conditions. Environmental factors are important influences on the allelopathic activity of Mexican sunflower in soil but Mexican

sunflower may be valuable for better weed management in sustainable agriculture in the uplands of northern Thailand.

Table 7.1 Physical and chemical characteristics of Hang Chut soil*, Lampang, Thailand, close to the area where Mexican sunflower has been grown (Ogawa *et al.*, 1980).

Soil depth (cm)	Texture	Total C %	Total N %	C/N	pH	CEC (me/100g)
0-17	Sandy loam	0.84	0.04	21.5	6.6	3.84
17-33	Sandy loam	0.42	0.03	14.5	6.8	3.52
33-65	Sandy loam	0.23	0.01	19.2	6.6	1.68
65-100	Sandy clay loam	0.19	0.02	10.6	6.0	3.12

*Hang Chut soil is the Gray Podzolic soil, brown black colored, fine granular, semi dry and well drained.

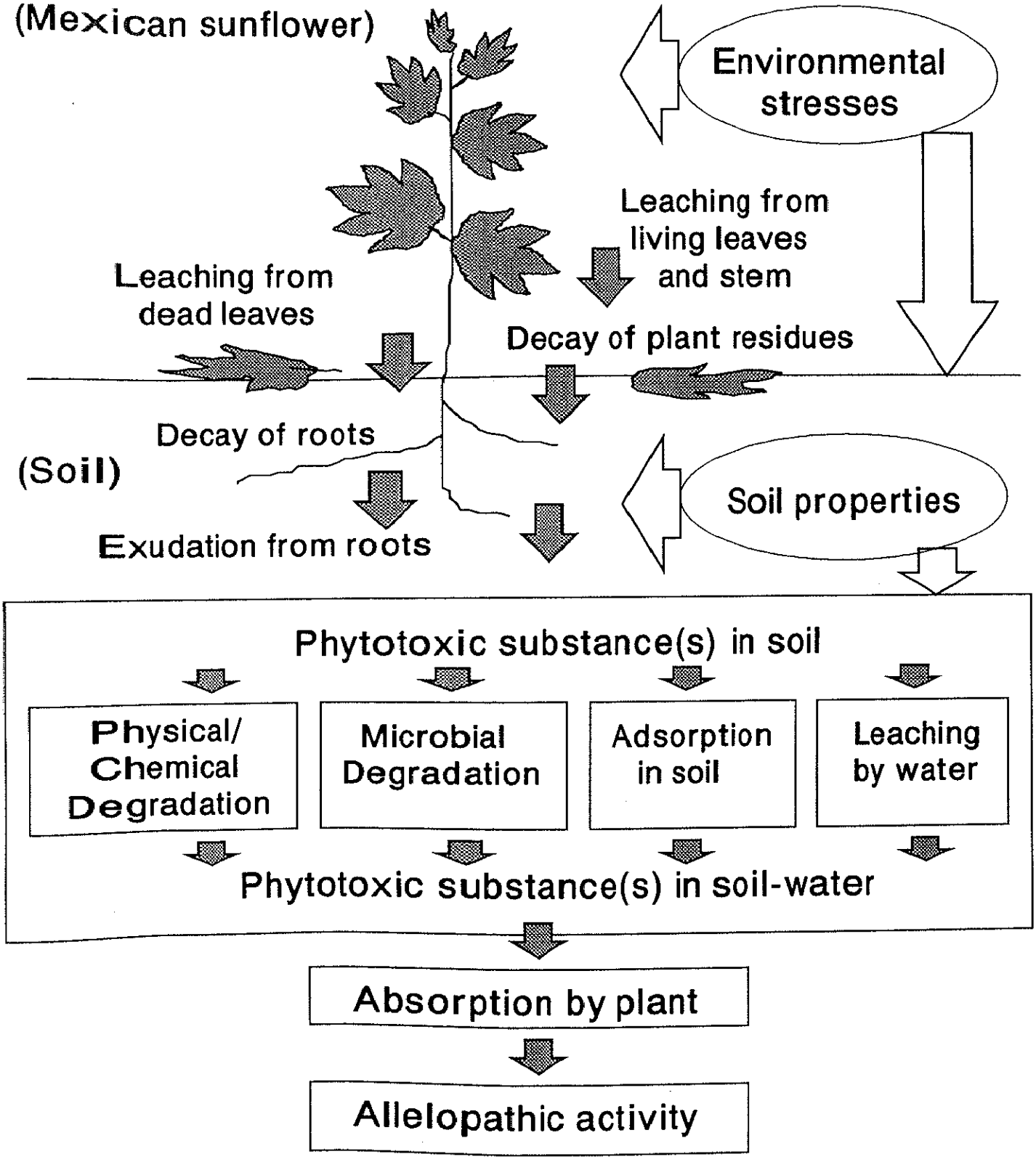

Fig.7.1 The proposed processes by which allelopathic activity is induced and the factors that affect the activity of phytotoxic substance(s) released from Mexican sunflower.